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Breeding to adapt agriculture to climate change: affordable phenotyping solutions

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Breeding is one of the central pillars of adaptation of crops to climate change. However, phenotyping is a key bottleneck that is limiting breeding efficiency. The awareness of phenotyping as a breeding limitation is not only sustained by the lack of adequate approaches, but also by the perception that phenotyping is an expensive activity. Phenotyping is not just dependent on the choice of appropriate traits and tools (e.g. sensors) but relies on how these tools are deployed on their carrying platforms, the speed and volume of data extraction and analysis (throughput), the handling of spatial variability and characterization of environmental conditions, and finally how all the information is integrated and processed. Affordable high throughput phenotyping aims to achieve reasonably priced solutions for all the components comprising the phenotyping pipeline. This mini-review will cover current and imminent solutions for all these components, from the increasing use of conventional digital RGB cameras, within the category of sensors, to open-access cloud-structured data processing and the use of smartphones. Emphasis will be placed on field phenotyping, which is really the main application for day-to-day phenotyping.

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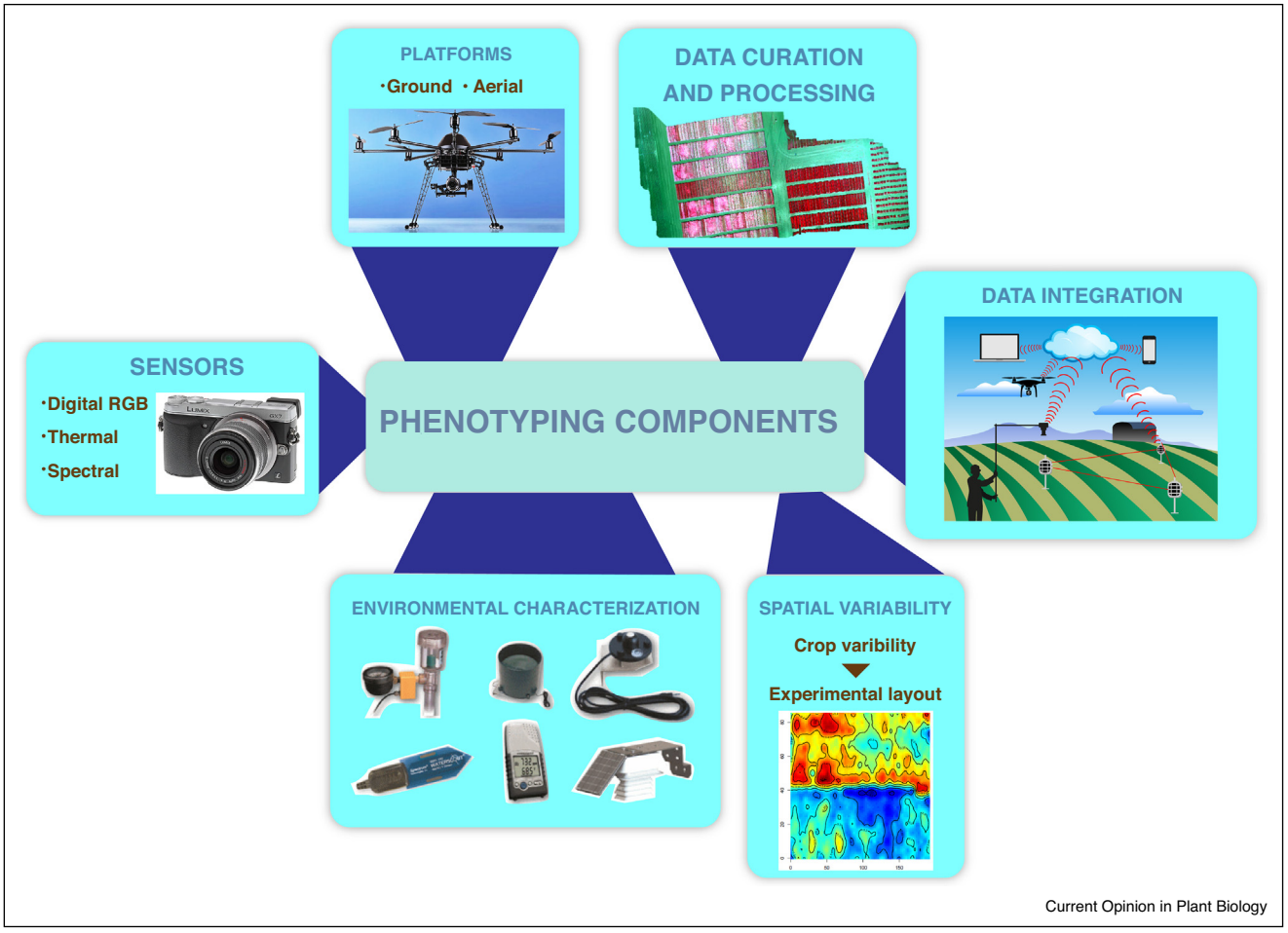
Introduction

Phenotyping is currently perceived as a bottleneck hindering the advance of crop breeding [1•]. Consequently, research into reliable high throughput phenotyping techniques has increased dramatically in recent years, including a very wide range of approaches ranging from the tools [2,3••,4] to the platforms needed to carry out phenotyping [5]. However, phenotyping is not just the use of the proper tools and their placement (i.e. platforms), but also includes the throughput of information processing, including how amenable the row data extraction and the proper algorithms are to convert data to the target traits. Moreover, when field phenotyping is the main objective, phenotyping also includes proper characterization of the environmental conditions and spatial variability of the trials (Figure 1). On top of that, the use of all this information in a coherent, albeit efficient manner in terms of breeding objectives (e.g. conventional breeding, MAS, genomic selection, etc.) needs substantial capacity for information processing. In that sense, the perception of phenotyping as an expensive exercise somehow prevents the full exploitation of its possibilities, or at least conditions the way in which it is approached and implemented. However, effective and expensive are not necessarily synonyms. The perception that something affordable or low cost is not reliable is not necessarily true concerning phenotyping. In fact for breeders, putting field phenotyping into practice frequently means choosing affordable approaches to measure 'proven traits'. Affordable approaches are already available and will become more so in the future, and this will be true for all components and domains embraced by phenotyping, including the physical tools, algorithms, software and the data processing environment. In what follows, affordable alternatives for these components are discussed.

Traits and sensors

Remote sensing techniques are currently the most commonly used approaches in crop phenotyping, given their versatility (in terms of deployment, targeted traits, and platforms used, etc.). In fact, low cost remote sensing has always been implicit in the phenotyping activities of breeders, with the lowest cost sensors being the breeder's eyes. This is the case for ranking through visual evaluation of key traits (e.g. plant vigour, stress tolerance, phenology). The potential advantage of 'modern' remote sensing approaches is that they produce standardized data that does not depend on the personal perception of the breeder. Increasing interest in low cost solutions is evidenced not only by the number of workshops being developed but also by the appearance of platforms that address the development of affordable tools (e.g. GOSH, http://openhardware.science/; PhotosynQ, https://photosynq.org/). Among the remote sensing approaches, multi/hyperspectral and thermal sensors, which also include imagers (or cameras), together with the conventional RGB (Red-Green-Blue) cameras (Figure 2), are by far the most commonly used [1•]. Affordable approaches exist for these three categories of sensors. Among thermal

Figure 1

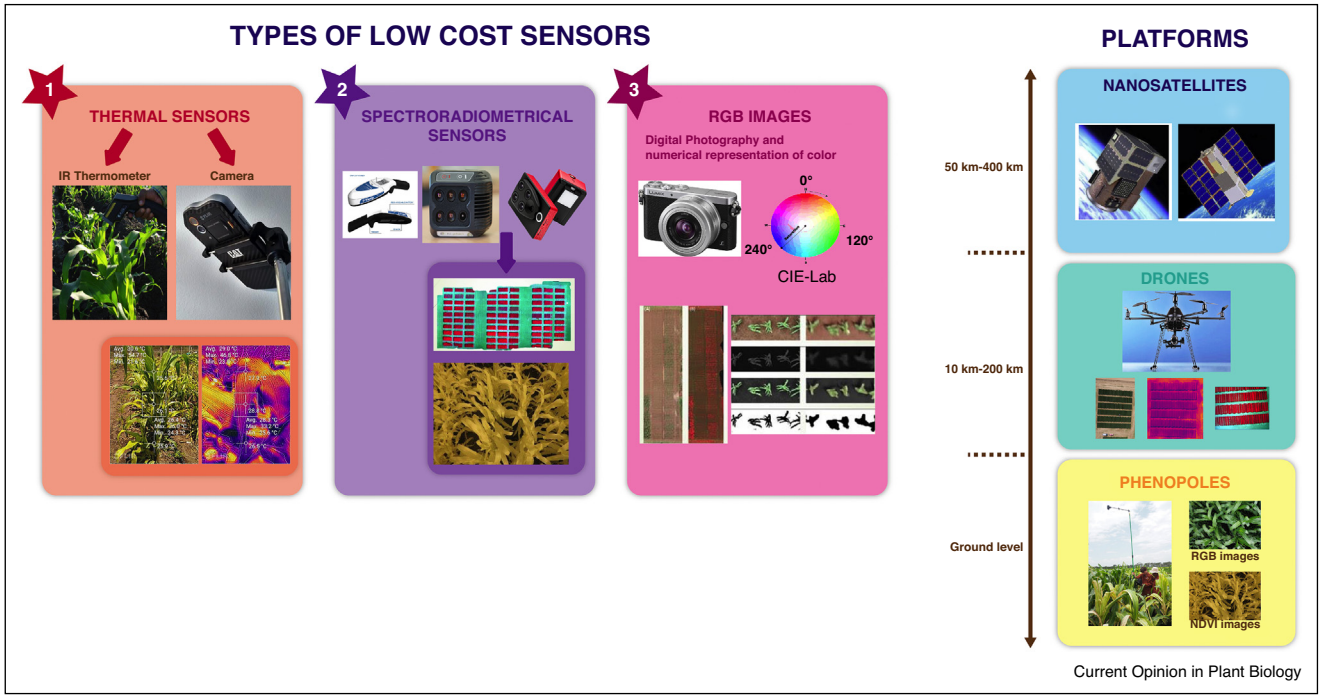


Affordable phenotyping is not just the choice of an appropriate low-cost sensor and platform. Phenotyping operations encompass other aspects where affordable solutions can be implemented. Thus the phenotyping process comprises sensors in the field alongside the eventual support of lab analyses where NIRS techniques have a key role. Besides a direct use for phenotyping, platforms may also be deployed to assess spatial variability of the testing site. Additionally, environmental characterization of the growing conditions is required. The raw data collected needs to be processed through different steps that depend on the technique used (conventional sensor or imager in the case of remote sensing techniques) and the level of evaluation (ground versus aerial). Once the data is processed, different categories of end products/traits (e.g. vegetation indices, temperature) are gathered. Further steps include applying the information in an empirical (i.e. statistically supported) or analytical (e.g. decision support system) manner to support different breeding scenarios (conventional breeding, marker assisted selection, genomic selection).

sensors, the non-contact infrared thermometers (the so called 'infrared guns') were first proposed as phenotyping tools more than four decades ago [6]. Use of the imaging version of these devices, known as 'thermal cameras', has been unviable until recently for small breeding programmes due to their prohibitive cost. However, the recent implementation of thermal sensors installed in mobile phones, even if still of a very low resolution, is promoting the use of this approach because thermal smartphones cost not much more than a normal smartphone, and even more importantly, the camera may integrate with a device that allows processing in 'the cloud' (as we will discuss below). Thus, new mobile phones that incorporate thermal infrared imaging directly (CatS60) or via a mobile phone attachment (FLIR One Pro) using ultra-compact FLIR Lepton cores have already been commercialized.

Among the spectroradiometrical devices available, affordable approaches are diverse. They range from spectroradiometers with active sensors, which, based on the measurement of bands in the visible and infrared regions of the spectrum, generate the Normalized Difference Vegetation Index (NDVI) and its variants (e.g. green NDVI, Red-Edge NDVI or SAVI), to the use of multispectral cameras that capture a few (ca. [2,3••,4,5]) wavelengths bands within the visible and infrared spectrum and may still allow the formulation of a wider range of spectral indices [7,8•,9,10]. In this category we may even include conventional cameras modified by removing

Figure 2



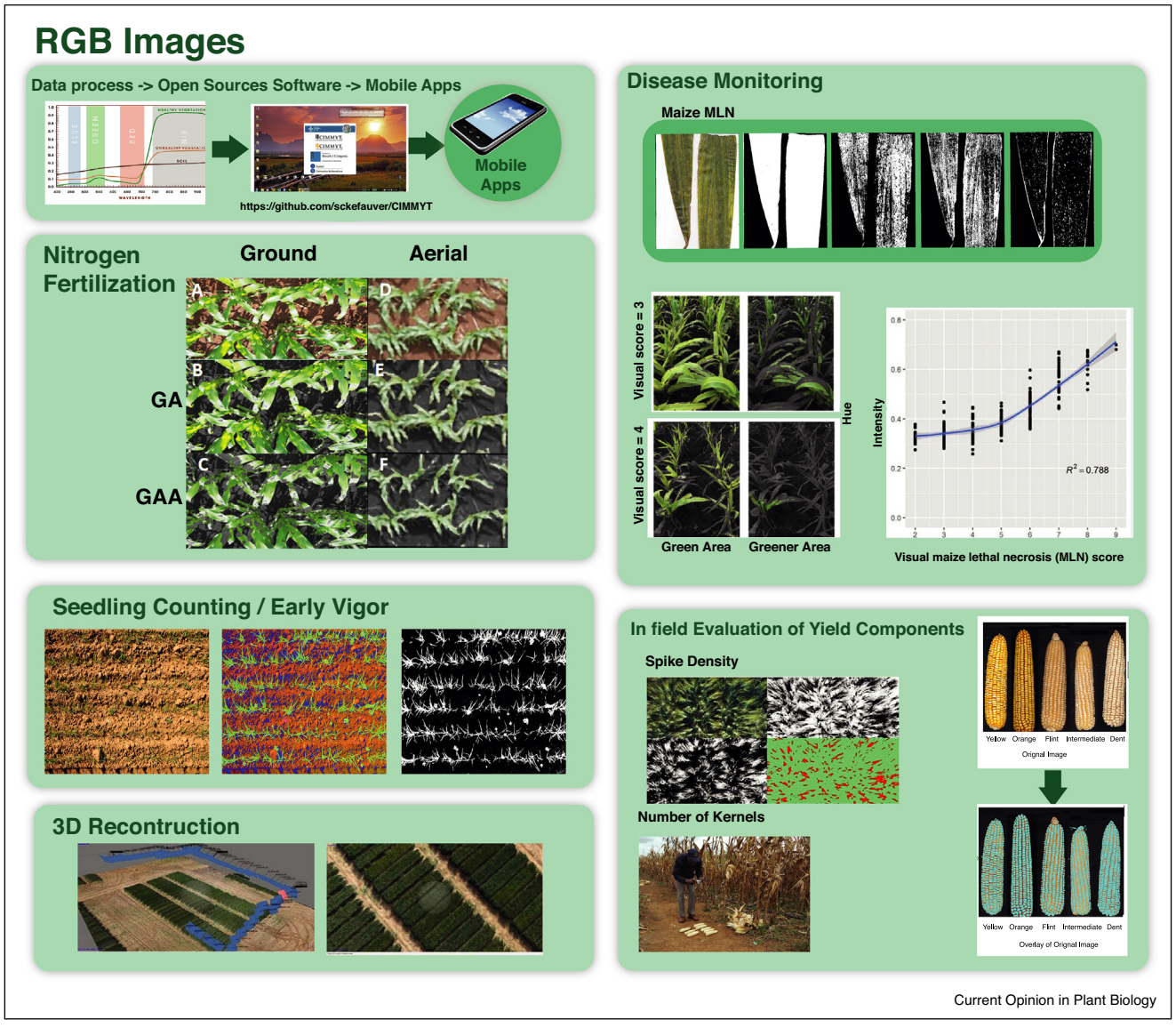
The remote sensing techniques most used in field phenotyping include thermal, spectroradiometric and RGB (Red-Green-Blue) sensors. For the first two categories, affordable options exist, either in the form of conventional sensors or even imagers (i.e. cameras), while RGB imaging is an intrinsically low-cost technique. The three techniques produce different categories of indices. Regarding platforms, aerial and ground are the two main categories. Among aerial platforms, unmanned aerial vehicles ('drones') are nowadays an affordable approach, and may be supplemented in the future with nanosatellites provided with high-resolution RGB cameras. In the case of ground platforms, carts and even more handheld devices, consisting of cameras or remotely controlled smartphones mounted on a pole, may represent a low cost flexible application.

the infrared blocking filters to assess the infrared region of the spectrum ([11]; https://publiclab.org/wiki/infragram-convertible-cameras).

RGB imagers: affordability and efficiency are linked

Conventional digital RGB cameras by themselves (i.e. without any modification) are the best examples of low cost sensors that deliver quality information. In fact, the use of RGB images allows a wide range of phenotyping applications and these will depend basically on the reliability of the algorithms implemented. This is one of the reasons behind the fact that a low-cost sensor, such as a digital RGB camera, is being included more and more as a key component in the most advanced phenotyping platforms, either under controlled [12] or field conditions. In the last case, RGB cameras can be installed in portable platforms on the ground (phenomobiles) [13] or aerially (e.g. unmanned aerial platforms) [5] and even in very sophisticated stationary platforms [14,15]. The RGB images are not just limited to the formulation of vegetation indices via open-access software [16•,17•] but allow a wide range of other applications (Figure 3). This is due to the wide versatility of the data (information) collected by RGB cameras, which is essentially linked to the high resolution of these images and the general high quality of factory colour calibration. For example, even though vegetation indices formulated using RGB images have a more limited spectral range and resolution (only broad bands within the visible region) than indices formulated by (multi and hyper) spectral sensors or imagers, the former generally have a much higher spatial resolution than spectral imagers. In fact, high spectral resolution can be substituted by high spatial resolution with equal or better results. The low cost of the approach may also ensure a higher temporal resolution (i.e. more frequent measurements) than the information provided by more expensive devices. High resolution may be even more important when phenotyping is performed from aerial platforms, such as UAVs, with conventional digital RGB photography that would ensure resolution down to sub-centimetre scale. For example, the Sony QX1 20 MP at 25 m altitude provides 0.2–0.5 cm resolution depending on the zoom, and most of the newer Sony Alpha series cameras offer 24–40 MP. Moreover, unlike RGB indices, spectroradiometrical indices like NDVI may be affected at longer (i.e. NIR) wavelengths by factors such as canopy architecture, which alters the reflectance properties of the plant, and mixed pixels including soil cover, especially at lower

Figure 3



Some examples of different applications of RGB images as affordable alternatives for field phenotyping, including crop monitoring and quantification of agronomical components. Visible images of single leaves, single plants or canopies are processed with open-access software (top left) that allows formulation of different vegetation indices for assessing crop responses to biotic (upper right) and abiotic (upper left) stress conditions. RGB images may also allow, through specific algorithms, assessment of soil cover and agronomical components such as stand counting (lower left), ear counting or grain counting (lower right). Three-dimensional reconstructions of individual plants or canopies (bottom left) are also possible using a single moving camera.

image resolutions [18]. In fact, there are increasing numbers of studies reporting that RGB indices outperform conventional spectral indices such as NDVI and others in a number of applications, including crop growth [19–21], evaluation of nitrogen [17•,22–24] and phosphorus status [25] or the impact of foliar diseases [26,27]. Limitations in RGB-derived information may arise in standardizing the light conditions, particularly in the field, when sunlight is used and atmospheric (presence of clouds, dust, etc.) conditions and daily/seasonal (sun angle and intensity) may eventually affect the throughput of the evaluation [13]. For this reason, it has been proposed that diffuse light may improve the quality of the assessment because it avoids the strong shadows and brightness caused by the reflection of direct sunlight (from epicuticular waxes). However, the potential impact of light conditions is not necessarily an overwhelming problem because it is often less relevant than expected (e.g. for wheat and barley assessed under Mediterranean conditions; unpublished results).

Besides the formulation of vegetation indices, RGB images are amenable for assessing a wide range of other traits in the field such as counting agronomical yield components [28••,29] (number of plants emerged, number of ears extruded, grain size), assessing phenological stages (heading, anthesis or maturity times), regular monitoring of crop development (e.g. soil cover, senescence), or even undertaking 3D assessments [30–35], of the growth of individual plants and canopies (plant height, biomass or the occurrence of lodging) (Figure 3). Other than the appropriate software (to run the specific algorithms), which is developing quickly, the main limitation is the need for high-performance computing capable of releasing results in near real-time. Solving that point may pave the way for a wide number of applications being implemented on the basis of RGB images alone.

Genetic gain can be improved by enhancing the heritability of selected traits. This implies appropriate monitoring of environmental factors and spatial heterogeneity at the field level [36,37,38•]. These low cost devices, particularly the RGB cameras and spectroradiometers, may also be used to assess spatial variability [1•,39]. High throughput approaches for root phenotyping, particularly under real (i.e. field) conditions, are not yet adequately validated in conventional phenotyping and this includes low-cost alternatives. In the case of maize and other crops, techniques such as 'shovelomics' [40,41], electrical methods and the measurements of brace root traits [41–43], even though promising, have not yet been fully proven. Besides other considerations RGB images may help to high throughput the 'shovelomics' approach.

When expensive sensors still matter

Near Infrared Reflectance Spectroscopy (NIRS) is a method used at the lab level for assessing chemical compositional traits related to, for example, plant quality and nutrition (nitrogen content, minerals, digestibility, amino acids, carbohydrates and others) [44–48]. While these traits may have a value by themselves as phenotyping traits, other chemical characteristics with potential as phenotypic traits may also be assessed through NIRS. This is the case, for example, of stable isotope signatures of carbon, nitrogen [49–51] and even oxygen [52]. In practical terms the strategy may be to use the spectra captured from samples during routine plant analyses (e.g. of nitrogen or mineral content) run through NIRS so that prediction models can be established for these other less common traits.

Hyperspectral reflectance is a very powerful tool that is capable of deployment in the field, even when the main limitation is the cost of the equipment [53]. It may allow screening of stable isotope signatures and photosynthetic parameters in a fully empirical (i.e. statistically driven) way, which otherwise would require destructive laboratory-based methods or time consuming gas-exchange measurements [54–57], or even prediction of final grain yield [53,58–61]. Similar to the NIRS big data techniques, hyperspectral reflectance may enable the building of robust prediction models that, for example, account for the effects of genotype × environment and wavelength × environment interactions incorporating genomic or pedigree information [62]. However, spectral signatures are influenced by many factors that include the external (e.g. light) conditions, which in the case of single leaves may be solved with the use of leaf clamps [58], and also leaf dorsoventrality. This factor must be taken into account, even in the isobilateral leaves that are typical of cereals [63].

Platforms

It is currently accepted that for high-throughput (precise) plant phenotyping it is not just the sensor but also the platform where the sensors are placed that has enabled advancements in this domain. Frequently, phenotyping platforms go a long way towards improving data capture quality by removing key bottlenecks related to mobility, data capture consistency and throughput, but they are often far from being low cost. In many cases, platforms have not exploited any major changes in remote sensor technology or processing beyond miniaturization. Upgrades in even the most expensive platforms have simply represented improvements in the mobility of existing remote sensing technologies, such as their adaptation to field phenotyping conditions [14,15].

Concerning platforms, even when unmanned aerial vehicles (UAV) have achieved increasing acceptance in terms of cost, reliability and technical requirements [64,65], regulations controlling UAVs in many countries still ban them or make them unfeasible in practice [66]. Affordable approaches using carts have been proposed, although their flexibility may be limited by the type of crop (e.g. tall crops such as mature maize, orchards) or even by its phenological stage [67]. The use of handheld platforms remains as the only low cost method, even though limitations might arise in terms of standardization and throughput [68]. In that sense the use of 'phenopoles' to attach cameras is a feasible option (Figure 2). While exposure and other settings in the camera may be remote controlled, a hand-held pole is amenable for reaching up to 4 m, which allows access, for example, to the top of the canopy in most crops and fruit trees.

Further progress in improving both sensors and platforms will evolve out of the rapid advances that are occurring in data processing. This will undoubtedly contribute to the popularization of mature, effective, low-cost, and high throughput plant phenotyping platforms [1•,3••,69,70].

Recent advances in sensor technology and the availability of free high-resolution (spatial and temporal) multispectral satellite images have afforded an opportunity to

forecast yield as well as mapping spatial distribution on a near real-time basis for regions where this was previously not feasible, such as Africa [71]. Nevertheless, at the present time satellite data is not yet mature enough to be used in traditional phenotyping platforms due to restricted pixel resolution. Thus, in a study at CIMMYT, NDVI has been derived from multispectral high-resolution satellite imagery for only relatively large sized plots [72•]. However, this study did not test vegetation indices derived from RGB-images. In fact, high-resolution RGB images may be even more amenable in the near future as phenotyping tools (Figure 3), and even more so when considering the deployment of nano/microsatellites (CubeSats) orbiting at quite low altitudes [73,74].

Smartphones as ground-phenotyping platforms

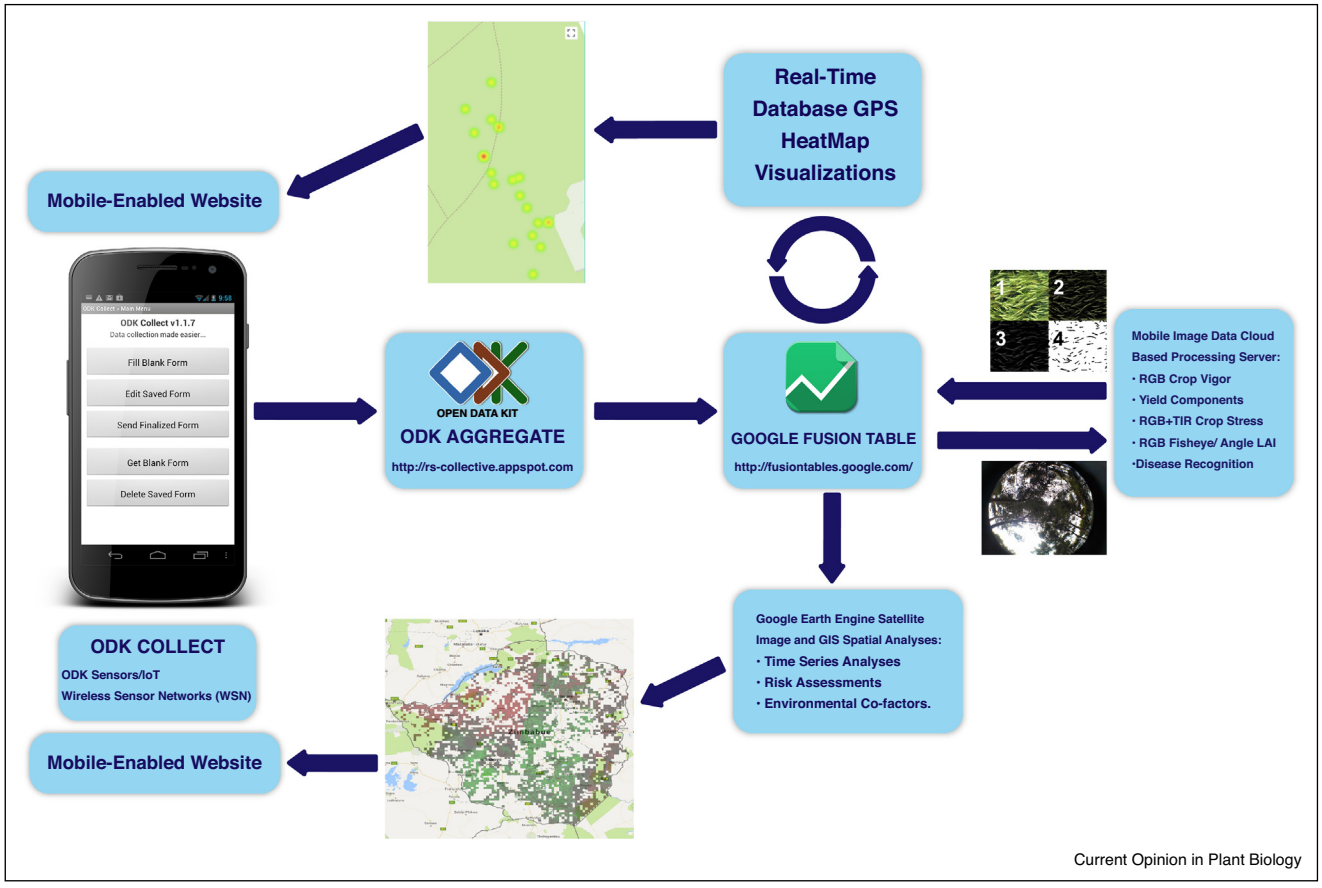
Agile phenotyping is being improved upon with complete mobile solutions, taking advantage of advances in sensor technology, algorithms and increases in computation power, in a single sensor-platform-processing solution, which is the modern smartphone. In this case, the camera-capture capacities of the mobile phone are combined with other built in sensors from the smartphone and processed in real-time using the smartphone's own processing capacities [75–78]. Integrated sensors on modern smartphones may include, but are not limited to, GPS geographic positioning, compass orientation, accelerometers, incident light sensors, and even thermal infrared imaging cameras. Simple calculations with RGB image data using alternate colour spaces and incident light provide accurate fractional vegetation cover estimates from above crop canopies [76,77], which is similar to the field photographic analyses of Breedpix [16•] and also comparable to the standard multispectral NDVI for assessing crop vigour and biomass. Going one step further, PocketLAI also integrates specific accelerometer and compass orientation capacities allowing for an accurate estimation of the Leaf Area Index (LAI) using images captured from underneath crop canopies with reduced computing requirements [79]. Other mobile apps offer more detailed and specific products based on image analyses by applying species-specific empirical relationships [80]. An array of other practical applications is already available such as mobile apps for rice leaf colour and embryo and endosperm analysis (https://www.youtube.com/watch?v=6NTjheFEsqE) or even fruit counting [81].

Moreover, in terms of a single sensor-platform-processing solution, smartphones are particularly amenable to working with networks of wireless sensors for environmental characterization, which is another component of the phenotyping process (Figure 1). A wide array of options is now available, for example to instantaneously integrate extensive environmental metadata with mobile phone-based image capture using either built-in or externally connected sensors [82–84]. These range from weather stations to soil sensors of different types (e.g. www.dynamax.com). Thus, low cost electrical conductivity sensors and an autonomous power supply based on energy harvesting may provide real time data of, for example, the salinity conditions of rice paddies (http://neurice.eu/wp-5-development-of-a-wireless-sensor-network/) and couple that with RGB and/or thermal images of crop conditions.

Data processing

Data collection as a bottleneck is no longer a concern. Nevertheless, in the age of big data, the problem of too much data needs new tools to overcome the data-to-decision barrier. Just like the increase in efficiency and quality of data capture from the latest high-throughput plant phenotyping systems, computer processing capacity and algorithms that previously struggled to keep pace are now even surpassing data capture speeds. As a result, processing capacity is supporting unique data analysis techniques that, although frequently based on known science, were previously impossible due to massive computing requirements [85]. The improved processing techniques using UAV RGB images, with 3D and plot segmentation tools, are just a couple of examples. In the case of 3D modelling, for automation of the quantification of crop structural data, using image parallax for estimating distances and heights between several points is routine in cartography, but doing the same to produce a 3D reconstruction based on millions of precisely modelled points has only been possible due to recent technological leaps in computer processing power. Furthermore, these 3D reconstructions benefit most from high-resolution visible light (RGB) images, which are readily available from the highly competitive commercial digital photography market at a relatively affordable price. Thus 3D modelling is usually undertaken with commercial software (e.g. Agisoft Photoscan Pro or Pix4D) and, although commercial software can be pricey, educational and research discounts can be generous. However, lower-cost alternatives exist including open-source software (e.g. VisualSFM, ImageJ, QGIS), or tailor-made proprietary software (Pheno-Copter, CSIRO, Australia https://phenocopter.csiro.au/) [5]. In the case of image segmentation for UAV plant phenotyping studies, many software options exist (e.g. semi-automatic software MosaicTool for UAV mosaic images; a University of Barcelona Plugin for FIJI). Once the images are processed, extraction of the relevant data is required. Within this domain, formulating data from vegetation indices is an example where open-access alternatives are available. This is the case for the CIMMYT MaizeScanner for RGB field-based phenotyping (available at http://github.com/sckefauver/CIMMYT). This FIJI plugin calculates a number of RGB-based indices for estimating disease impacts, crop vigour, LAI, and biomass at the leaf and canopy scale including Breedpix (GA and GGA), the Triangle Greenness Index

Figure 4



An affordable and accessible method for integration of mobile phone phenotyping is illustrated. Data from the smartphone is uploaded and compiled via a remotely managed aggregate server using the Open Data Kit (ODK). ODK provides accessibility to smartphone deployment for data collection by providing users with the mobile app structural framework, which requires only a custom spreadsheet or xml query file in order to be deployed for data collection and compilation of user data to Google Fusion Tables. Then, the online database can easily be shared, queried, visualized, or accessed by cloud based processing servers or used as inputs to the Google Earth Engine satellite processing platform, with results providing user feedback and/or guidance through mobile-enabled web portals to provide real-time 'heat map' style data visualizations. As examples, mobile RGB images may provide general crop vigour or water stress (in the case of RGB + TIR) indicators with in situ processing. Alternatively, mobile phones may be used to implement more advanced processing algorithms and/or image capture techniques, which are then processed on a remote server that subsequently updates the online database in near real-time. Such examples may include yield component segmentation algorithms and using hemispherical lens adaptations or mobile phone accelerometers for specific angles (as implemented in PocketLAI) to provide more specific crop biophysical parameter retrieval such as Leaf Area Index (LAI). Additionally, other computationally intensive A.I. (artificial intelligence) or machine learning approaches to disease classification and quantification may be moved to more powerful cloud based processing in order to reduce battery drain in the field. Exceptional documentation on the ODK to Google Fusion Table process is provided for instruction, while the separate data processing servers and Google Earth Engine code must be tailored to specific projects. However, support and development for both are active, with planned upgrades with ODK Sensors (http://opendatakit.org/use/sensors/) for integrating with wireless sensor networks (WSNs) and the Internet of Things (IoT), which will greatly enhance metadata possibilities while also reducing dependence on lengthy ODK user forms.

(TGI), and the Normalized Green Red Difference Index (NGRDI) [86].

Phenotyping integration and smartphones

Smartphones can now connect to a variety of external sensors over wired and wireless channels. Therefore, another approach to the use of mobile phones focuses less on the capacities for real-time data processing, which can have significant negative impacts on battery life, and instead take advantage of smartphone connectivity [87••] via WiFi and mobile data networks (4G, 3G, EDGE, etc.) so as to upload and compile data via a remotely managed aggregate server (Figure 4) such as the Open Data Kit (ODK) project [88,89]. ODK has the specific advantage of accessibility because the user need only create a form to which mobile app users can then add data via text from other sensors, use the mobile phone's integrated sensors, or even incorporate data through an associated wireless sensor network [89,90]. The ODK system (https://opendatakit.org/use/sensors/) has the distinct advantage

of having been developed in collaboration with Google, enabling the low-cost deployment of an aggregate sever for data compilation to a Google Fusion Table, and even subsequent connection to the Google Earth Engine satellite processing platform (Supplementary Fig. S1). This enables the use of ODK data in direct satellite analyses for relevant research [91–93]. Other alternatives include open-source applications, such as MultispeQ Beta, which allows data to be shared on a large scale through mobile devices and laptops [94]. In summary, there is a demand for the further development of smartphone-based approaches for different purposes. However, ensuring proper device interaction can be burdensome, especially when a single application needs to integrate with a number of sensors using different communication channels [89]. In fact, mobile phone applications connected to cloud-based computer processing have room for improvement. Nevertheless, this will become less of an issue with the imminent release of 5G, with gigabit data throughput, making data transfer near instantaneous and the need for local processing obsolete. Once the data is in the cloud it can be processed in minutes instead of hours or days.

Conclusions and way ahead

Advances in sensors, platforms and processing have brought image analysis to the forefront of HTPP research via a rapid growth in the number of potential applications for airborne and mobile imaging. The availability and effectiveness of affordable plant phenotyping sensors has recently experienced a revolution in terms of cost per imaging capacity and imaging capacity per sensor size. These two key advances, driven in part by the boom in mobile phone technology, have resulted in more affordable, more powerful and yet smaller sensors. Although they are mostly limited to the visible RGB range, they also including some near-infrared and thermal sensors that can be integrated into an increasing number of sensor platforms. Indeed, as sensors become smaller and lighter they can be deployed on a greater number and variety of phenotyping platforms. Moreover, over the coming years, microsatellites mounted with high-resolution RGB cameras may become a competitor to aerial phenotyping platforms. The benefits of improved integration between sensor and platform has enabled the development of full phenotyping systems with the ability to capture, combine and store data for multiple different subsystems simultaneously in an organized, efficient and affordable manner. The result for the field of plant phenotyping, where massive data collection has long been a major bottleneck, is a revolutionary increase in the agility of phenotyping systems.

There are still challenges in the implementation of phenotyping as a whole and of low-cost approaches in particular. These include image segmentation in the field, and integration of concepts mentioned above such as 3D, thermal multispectral, and hyperspatial mobile apps, and big data, including deep learning and machine learning. On top of that, efficient decision-making (by decision support systems) is regarded as a necessary innovation to support the practical phenotyping that is yet be developed. To conclude, adoption of HTPP by breeders will only be achieved if it is demonstrated as something valuable in terms of genetic gains attained versus resources invested [95]. It is in this context that affordable crop phenotyping becomes a strategic choice for breeding.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article https://can be found, in the online version, at doi.org/10.1016/j.pbi.2018.05.003.

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- •• of outstanding interest

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